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RESEARCH ARTICLE

Physiological and biochemical changes during seed deterioration in *Perilla frutescens* (L.) Brittion

VISHAL SINGH AND VEENA GUPTA

SUMMARY

Accelerated ageing test was conducted in laboratory on seeds of 20 different accessions of *Perilla frutescens* (L.) Brittion to understand the effect of deterioration on seed health and viability. Seeds were aged for 48 and 72 hours though accelerated ageing treatment (100% RH and 44^0 C temperature) and were analyzed for various physiological and biochemical parameters. The germination percentage, vigour index, speed of germination and seedling length decreased as the accelerated ageing period increase in all seed lots. Average increase in the electrical conductivity values of seed leachates over control was 106.55 per cent after 48 hrs and 170.27 per cent after72 hrs of ageing treatment. Total soluble protein declined by 30.83 per cent after 48 hrs and 50.34 per cent after 72 hrs of accelerated ageing. Thousand seed weight was positively correlated with speed of germination and germination percentage and was negatively correlated with lipid peroxidation and decrease in soluble proteins. Lipid peroxidation values increased significantly in aged seed lots over the control.

Key Words **:** Seed ageing, Germination, Seed vigour, Protein profile, Lipid per oxidation

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Perilla frutescens (L.) Brittion is an important underutilized, multipurpose, traditional crop of India, China, Japan. Korea, Thailand and other Asian countries. In India, *Perilla* is mainly grown as a minor oilseed crop in Himalayan highlands and requires adequate research, marketing and conservation interventions as many of these underutilized crops are now drawing increase attention of policy maker world wide for their nutritional and other desirable traits. The seed oil is used for cooking, as a drying oil and as a fuel. The seeds are eaten by people and used as bird seed. The foliage is used as a potherb, for medicine and for food colouring. Limited information is available on the metabolic changes that occur during seed deterioration.

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
VISHAL SINGH, National Bureau of Plant Genetic Resources, Pusa Campus, NEW DELHI, INDIA
Email: veena@nbpgr.ernet.in

Address of the co-authors:
VEENA GUPTA, National Bureau of Plant Genetic Resources, Pusa Campus, NEW DELHI, INDIA

MATERIALS AND METHODS

Freshly harvested seeds of twenty accessions of *Perilla frutescens* after material and metheds were procured from the N.B.P.G.R, New Delhi (collected during NATP project by different NBPGR regional stations and grown at Shillong station) for comparative study on various physiological and biochemical parameters.

Each of the twenty accession seeds were divided into three lots and evaluated for various important physiological and biochemical parameters after giving accelerated ageing treatment (44^0 C temperature and 100% relative humidity) for 48 and 72 h. Untreated seed stored at ambient conditions served as control. The aged seeds were air dried under ambient conditions in moisture content laboratory and were analyzed for various physiological and biochemical parameters as follows.

Moisture content estimation was done by gravimetric method (Low constant temperature oven method, at 103^{0}C for 16 hr) as per ISTA Rules (ISTA, 1993).

Material used in the study		
Sr. No.	Name of accessions	Region of collection
1.	IC-281713	Uttarakhand
2.	IC-521292	Uttarakhand
3.	IC-521283	Uttarakhand
4.	IC-538084	Uttarakhand
5.	IC-003865	Meghalaya
6.	IC-003708	Meghalaya
7.	IC-521289	Uttarakhand
8.	IC-538007	Uttarakhand
9.	IC-521287	Uttarakhand
10.	IC-369349	Uttarakhand
11.	IC-521282	Uttarakhand
12.	IC-361361	Uttarakhand
13.	IC-419564	Uttarakhand
14.	IC-419568	Uttarakhand
15.	IC-526643	Meghalaya
16.	IC-526686	Meghalaya
17.	IC-528684	Uttarakhand
18.	IC-335408	Uttarakhand
19.	IC-526419	Meghalaya
20.	IC-526719	Meghalaya

Germination percentage :

Fifty seeds in two replications of each were plated between the towel papers and incubated in a germinator maintained at a constant temperature of 25^{0}C. Seeds were considered germinated when 1 mm radical emerged. The germination tests were evaluated on the 7th days of planting (ISTA, 1993). For speed of germination two replication of 50 seeds each were plated and the germinated seeds were removed every day from the sample. The speed of germination was calculated by the following formula (Maguire, 1962).

$$\textbf{Speed of germination} = \sum \mathbf{n/t}$$

where, n=no. of newly germinated seeds at time t

Vigour index:

Vigour index was calculated as the product of seedling length (root + shoot length) and germination percentage (Abdul Baki and Anderson, 1973).

Vigour index = Germination % X (shoot length + root length)

Electrical conductivity of seed leachate :

Twenty-five undamaged seeds were weighed in three replicates each and soaked in 25 ml of deionized water at room temperature (25^{0}C) for 17 h. in the dark. The conductivity of resultant leachate was measured after 17 h using conductivity meter (Control Dynamics, India). The readings were recorded in mS'/cm/g of seed.

Soluble proteins estimation :

Seeds were extracted in 0.01M phosphate buffer (pH 7), precipitated in 10 per cent TCA (Trichloro-acetic acid) re-dissolved in 0.1N NAOH and estimated by Lowry's method (Lowry *et al.,* 1951).

Lipid peroxidation :

Lipid peroxidation was measured using TBA – TCA reagent (0.5% Thiobarbituric acid in 20% trichloroacetic acid) following the method of Heath and Parker (1968). A sample of 0.5 g of seed material was homogenized in 5 ml of distilled water and 5 ml of TBA – TCA reagent. These samples were incubated at 90^{0}C for 30 minutes in capped reaction tubes. After incubation the samples were cooled in ice bath and centrifuged at 5000 rpm for 10 min. The OD of the supernatant measured at 535 and 600 nm. After subtracting the absorbance of nonspecific (620 nm) from specific (535 nm) the net absorbance was expressed in terms of absorbance at 535 nm/ mg fresh wt., which indicated the level of malonealdehyde (MDA) produced as a result of lipid peroxidation.

Statistical analysis :

The data from the laboratory experiments were analyzed statistically by adopting CRD technique (Panse and Sukatme, 1985). The values in percentage were converted into Arcsine values before analysis. Correlation analysis was done to see the association in trends of change in different parameters.

RESULTS AND DISCUSSION

Untreated seeds of all accessions germinated within two days but the ability to germinate showed gradual decline in aged seeds. Significant differences (at P = 0.05) in the values of germination per cent among different accessions were observed. In control samples minimum germination 53 per cent was observed in IC-369349. After 48 hours of accelerated ageing treatment IC-526643 showed minimum germination percentage of 11per cent.

Speed of germination :

The speed of germination is indicative of seed vigour. Significant differences were observed among seeds treated for different durations. The maximum value 11.76 and 11.45 was observed in control samples (IC-361361 and IC-538084) which subsequently declined to 0.22 and 3.88, respectively with time. The lowest value of 4.35 was observed for IC-369349 in control which declined to 0.22 after 72 hours of accelerated ageing. In general the control seeds had the high values of speed of germination in contrast with the very low or nil values at the highly deteriorated conditions. Storage under highly deteriorated conditions resulted in a significant fall in the vigour index among all deteriorated samples. Maximum vigour index in control samples was shown by IC-526686 (822) and minimum was for IC-369349 (257.59) (Fig. 1).

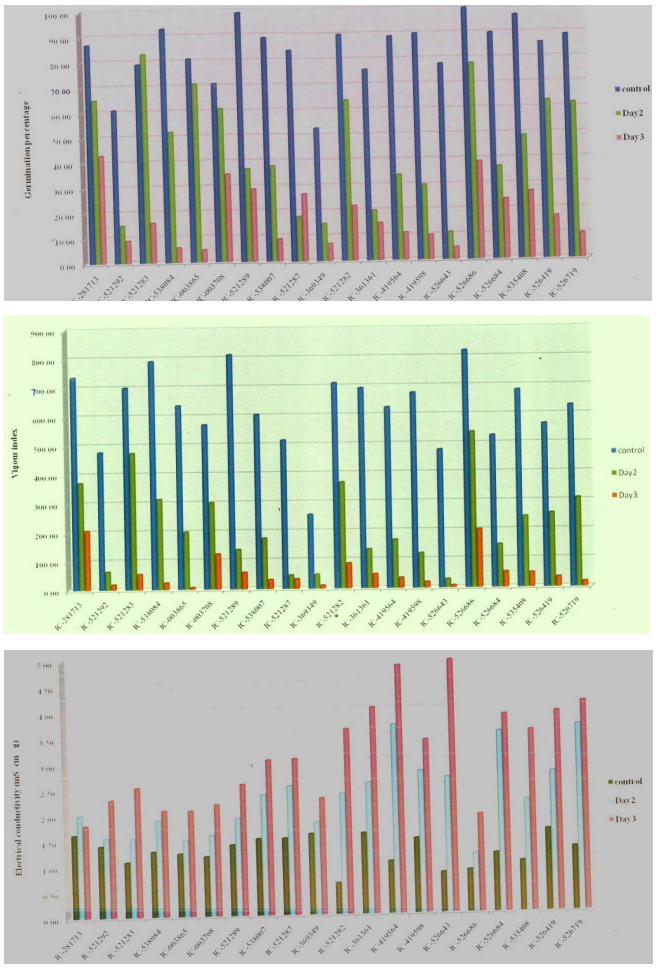


Fig. 1: Effect of ageing on seed quality parameters in *Perilla frutescens* accessions

EC of leachate :

The EC of leachate observed under different duration of accelerated ageing treatment increased significantly with increase in the level of deterioration. Per cent increase in the EC value ranged from 11.11 per cent to 519.48 per cent. The maximum conductivity of leachate was observed in the seeds aged for 72 hours. Average value of EC in control samples was 1.25 m/S/cm/min, 2.39 mS/cm/min in samples treated for 48h and was 3.088 mS/cm/min in the samples treated for 72h. This showed increasing pattern of change in EC value with increased level of deterioration (Fig. 1).

Lipid peroxidation :

Lipid peroxidation was analysed at different days of ageing treatment of seeds under high relative humidity and high temperature conditions. Lipid peroxidation values were the least in healthy and 100 per cent viable seeds but showed an increasing trend with seed deterioration. A significant increase of 3-4 times in the level of lipid peroxidation was recorded at high humidity and high temperature conditions of accelerated ageing. The highest value of lipid peroxidation after 72 hours of ageing treatment was shown by IC-526643. The maximum increase in lipid peroxidation value was shown by IC-526684, from 0.08 in control sample to 0.38 after 72 hours of deterioration.

Soluble proteins :

In general, the value of protein significantly decreased with period of ageing treatment in all the accessions. Average amount of soluble protein in the fresh (control) samples was 24.36 mg/g, which declined to 17.18 mg/g after 48 hours of accelerated ageing treatment and further declined to 12.53 mg/g after 72 hours of ageing treatment. IC-526686 was with maximum amount of soluble protein (34.58 mg/g) while IC-526719 was with minimum soluble protein (10.91 mg/g) in fresh samples.

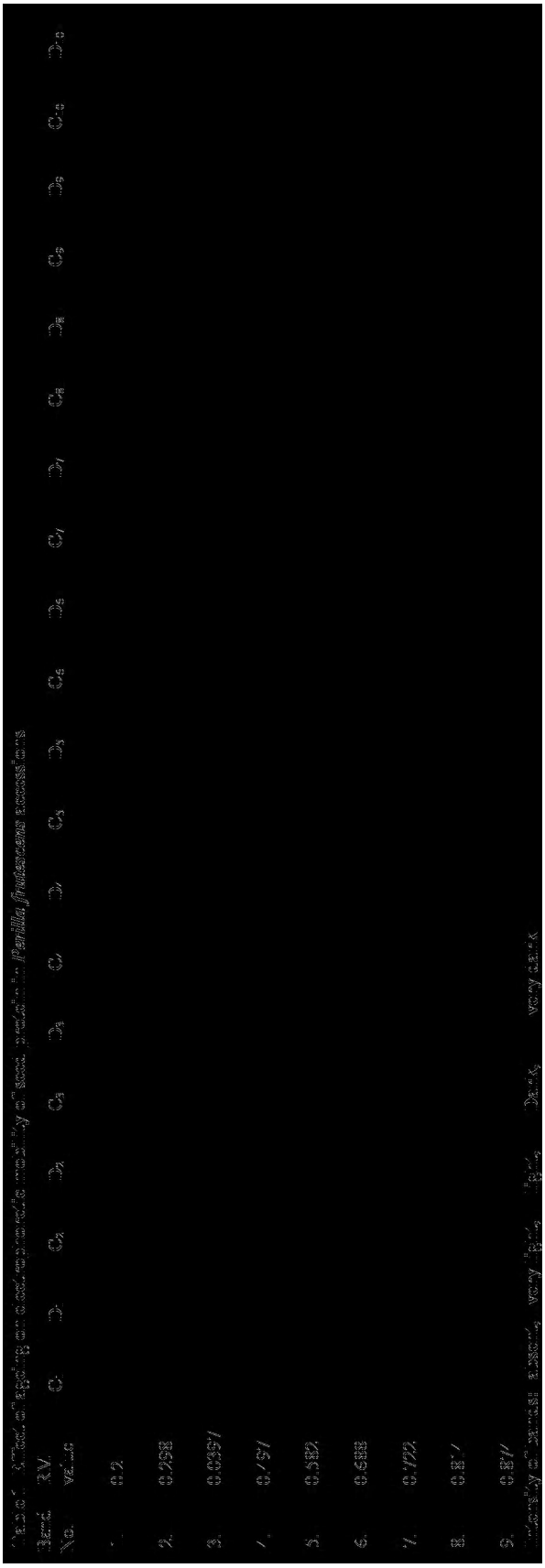
Correction analysis :

The correlation coefficients between different parameters after standardization of their values were calculated to assess any association between changes in different parameters across the accessions. After 48 and 72 hours of accelerated ageing reduction in germination percentage was significantly correlated with reduction in speed of germination (0.650). Thousand seed weight also showed significant negative correlation with the germination reduction (-0.754) and reduction in speed of germination (-0.679) and negative but non-significant correlation with other parameters which used to increase after deterioration such as increase in lipid peroxidation values and reduction in seedling length. Thousand seed weight was negatively correlated with decrease in soluble protein content.

Protein profile :

The protein profile of the *Perilla* seeds treated with accelerated ageing treatment showed a changed banding with increase in storage period. A band showing low mobility (high molecular weight protein) with Rm value 0.0397 showed decrease in the concentration in sample D_1 and D_4 over their control C_1 and C_4, respectively (Table 1). Band showing high mobility (lower molecular weight protein) with Rm value 0.722 and 0.814 were absent in aged samples (D_1, D_4) when compared with their respective control samples (C_1, C_4). Band with Rm value 0.874 showed absence in aged sample (D_1, D_4 and D_7) while it was present in their corresponding control samples (C_1, C_4 and C_7, respectively).

Seed deterioration under natural as well as storage conditions usually results in the loss of vigour and viability. In most of the seeds, the germination percentage decreases with ageing. The loss of viability is attributed to membrane permeability and biochemical changes. During deterioration, membrane in the seed axis undergoes changes which interfere



with membrane reorganization or with membrane stretching and repair during initial imbibition (Stewart and Bewley, 1980). In general, controlled ageing affects the germination capacity of the seeds adversely. In the present study, when all the twenty accessions of *Perilla* subjected to the deterioration through accelerated ageing treatment, each accession responded differently to the same condition (100% relative humidity and 44^{0}C temperature). Some accessions deteriorated faster when compared to other varieties expressing themselves as a poor storer. The maximum reduction in germination showed by IC-003865 (93.845% over control in 72 hours). Even though some accessions showed resistance to deterioration, the trend germination percentage was there with increasing controlled deterioration period. In all the cases of seed material, the percentage of germination was higher for fresh seeds. With increase in controlled deterioration period, the germination decreased in all the cases. This clearly demonstrates that negative influence of higher temperature and moisture on seed health and viability.

Seeds lots having high laboratory germination revealed large differences in their ability to emerge in the field (Mathews, 1980). Thus, laboratory germination is not a good indicator of field emergence potential. Vigour index decreased significantly in all the 20 accessions with increased duration of ageing treatment. It was maximum for control and minimum for seeds treated for 72 hours. Wide variation in the vigour index values among different accessions showed differential level of tolerance against ageing in different accessions. Loss in seedling vigour is reported to proceed with loss of seed viability in a number of crops (Harrington, 1972; Dey and Basu, 1982; Yadav *et al*., 1987; Dharamlingam and Basu, 1990). In the present study, decline in seedling vigour proceeded with lowering speed of germination concomitant with the reduction in germination which is in conformity with the earlier results of Raghuveer Rao (1988).Similarly ageing induced loss of vigour has been reported by Dey and Mukerjee (1988) in artificially aged mustard seeds.

Membrane is the most important site of a seed which appears to be adversely affected by seed deterioration / ageing degradative changes. This results in enhanced solute leakage from imbibed seed there by resulting in loss of viability and seed vigour .In the present study also, increase in electrolyte leakage was observed with the reduction in germination over ageing treatment time under all the accessions which is in confirmation with the earlier reports of Nutile, (1964), Powell and Matthews (1977), Halder *et al.* (1981). The increase in the amount of electrolytes was found to be proportional to the seed deterioration, attaining maximum values when seeds were highly deteriorated after 72 hours of accelerated ageing treatment.

Perilla seeds with time exhibited a declining trend in the quantities of total soluble proteins which are similar with the earlier reports of Nautiyal and Purohit (1985). The depletion of essential metabolites including loss of food reserves as one of the intrinsic theories of loss of seed viability. The decrease in level of proteins with increasing ageing treatment duration could be due to the increased denaturation of proteins during ageing. In addition, it is well demonstrated that free radicals produced during deterioration are the results of lipid auto oxidation, which is responsible for the denaturation

of proteins and loss of germination, which is directly related to enzyme activity of seeds. It might have more pronounced effect under higher temperature treatments (Grabe, 1964). This is also in agreement with the results of groundnut where both protein and oil content were lowered in storage leading to the increase in free fatty acids, accompanied by a loss in viability (Ramamoorthy and Kaarivartharaju, 1986).

Lipid peroxidation :

The increase in malone aldehyde throughout the levels of ageing in seeds was reported by several workers (Herman and Mattick, 1976; Stewart and Bewley, 1980). Under the present studies there was a progressive increase in the lipid peroxidation during accelerated ageing in *Perilla* seeds.Lipid peroxidation produces highly reactive free radical intermediates that can damage membranes, proteins and nucleic acids which were observed to precede the loss of viability in *Quercus ruber* axes (Finch-Savage, 1992). The free radicals accumulate because the scavenging systems are not very effective in the dehydrated state. Since free fatty acids cause membrane fusions at all moisture levels regardless of the presence of the protectants they are considered to destabilizing the membrane structure. While lipids are particularly susceptible to peroxidative attack and the by products of these reactions also affect protein and nucleic acid function (Witz, 1989). These studies indicated a good correlation of increased electrolyte leakage with increased lipid peroxidation content, which indicates the destruction of membranes caused by peroxidation of lipids during desiccation. Changes in the membrane integrity (increase in EC values, lipid peroxidation) and lowering of protein values in aged seeds of *Perilla* species might be the possible reason for loss in seed viability during accelerated ageing at high temperature and high humidity.

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